Pest Management Grants Final Report

Contract No. 00-0215S

Use of a Natural Product to Stimulate Sclerotial Germination of *Sclerotium cepivorum* for the Control White Rot of Onions and Garlic

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Abstract

White rot of onion and garlic, caused by the soilborne fungus *Sclerotium cepivorum*, is a worldwide threat to *Allium* production. No economical control measures currently exist and once a field is infested, it will remain so indefinitely since sclerotia of the fungus remain dormant in the absence of *Allium* plants. Hence, infested fields are often forever abandoned from further onion or garlic production. Sclerotia germinate only in response to root exudation of specific volatile sulfides and thiols. If these sulfides can be applied to the ground in the absence of an *Allium* crop, the sclerotia may be "tricked" into germinating. In the absence of a host, the mycelium from germinating sclerotia persist for a while, then die after exhausting nutrient reserves. In this study, garlic powder and synthetic garlic oil were incorporated by split application into the soil by deep plowing and mulching. Methyl bromide fumigation was applied once under tarp. Periodically after treatment, the number of viable sclerotia in the soil dropped significantly in the treated plots, approaching the degree of sclerotia mortality achieved with an application of methyl bromide. Diallyl disulfide was more effective in reducing sclerotia populations than garlic powder. The amount of white rot in garlic planted in the fall, 2001, was determined in May, 2002, by visual evaluation of symptoms and by measuring yields in each plot. Relative to the untreated control, white rot symptoms were reduced and yields were increased by DADS and methyl bromide. Garlic powder did not reduce the incidence of white rot.

Executive Summary

White rot of onion and garlic, caused by the soilborne fungus *Sclerotium cepivorum*, is a worldwide threat to *Allium* production. No economical control measures currently exist and once a field is infested, it will remain so indefinitely since sclerotia of the fungus remain dormant in the absence of *Allium* plants. Hence, infested fields are often forever abandoned from further onion or garlic production. Sclerotia germinate only in response to root exudation of specific volatile sulfides and thiols. If these sulfides can be applied to the ground in the absence of an *Allium* crop, the sclerotia may be "tricked" into germinating. In the absence of a host, the mycelium from germinating sclerotia persist for a while, then die after exhausting nutrient reserves. In this study, garlic powder and synthetic garlic oil (DADS) were incorporated into the soil in split applications. Periodically thereafter, the number of viable sclerotia in the soil dropped significantly in the treated plots, approaching the degree of sclerotia mortality achieved with an application of methyl bromide. Garlic was planted in the plots one year after treatment; white rot development was evaluated in May, 2002. Relative to the untreated control, white rot symptoms were reduced and yields were increased by DADS and methyl bromide. Garlic powder did not reduce the incidence of white rot.

Body of Report

Introduction. White rot of onion and garlic, caused by the soilborne fungus *Sclerotium cepivorum* Berk, is a worldwide threat to *Allium* production. The disease is extremely serious on these crops - an inoculum density of a single sclerotium in a liter of field soil can potentially result in crop failure and no economical control measures currently exist. Furthermore, once a field is infested, it will remain so for at least 40 years and probably longer since sclerotia of the fungus remain dormant indefinitely in the absence of *Allium* plants. Loss estimates to this disease are difficult to ascertain because once identified in a field, growers are forced to grow other, nonsusceptible (non-*Allium*) crops. Hence, infested fields are often forever abandoned from further onion or garlic production.

The white rot fungus produces no functional spores. Instead, it propagates only by the production of round, poppy seed-sized sclerotia produced on the roots of decayed host plants. Sclerotia spread in mass movement of soil or water, on animals (at least theoretically), and especially on infested plant parts. Once introduced into an area, *S. cepivorum* is gradually spread on contaminated equipment or planting materials, and slowly the production of garlic and onions in the entire region is threatened. Garlic culture is perhaps the principal mode of movement since it is propagated vegetatively, and garlic bulbs and cloves are sufficiently large that an infestation might go unnoticed. In any case, the disease is spreading throughout western North America.

Traditional methods to control white rot are either economically prohibitive or ineffective. Currently, the only effective method of control is tarped fumigation with methyl bromide. This method may kill 99% or more sclerotia in the soil, but does not result in complete eradication. Therefore, retreatment has to made on an ongoing basis since very few viable sclerotia remaining in a field can result in disastrous consequences in *Allium* production. Because retreatment with methyl bromide may be necessary, its use may not be cost effective. Moreover, the material itself is scheduled to be phased out for use in a few years according to the U.S. Environmental Protection Agency, which initiated action under the Clean Air Act for a phase out of chemicals threatening the ozone layer by the year 2005.

White rot is a disease limited to *Allium* crops. The fungus successfully colonizes only *Allium* plants and sclerotia germinate only in response to exudation by *Allium* roots. These exudates contain alkyl and alkenyl-L-cysteine sulphoxides, which are metabolized by the soil microflora to yield a range of volatile thiols and sulfides that activate the dormant sclerotia. The specific reaction between sclerotia and sulphoxides or their breakdown products suggests a possible use of these sclerotial germination stimulants for controlling white rot disease. If these thiols can be applied to the ground in the absence of an *Allium* crop, the sclerotia may be "tricked" into germinating. In the absence of a host, the mycelium from germinating sclerotia persist for periods ranging from a few days to several weeks depending on the soil temperature, then die after exhausting nutrient reserves.

One natural sclerotial stimulant from *Allium* spp. is diallyl disulfide, which is also recoverable from the distillation of petroleum. Recent research demonstrated that diallyl disulfide (DADS) distributed through the soil profile in the absence of an *Allium* crop in an infested field forced 90-99% of the sclerotia to germinate. This degree of germination resulted in disease control that is similar to control achieved with methyl bromide fumigation

Garlic powder, a deregulated product from dehydrated garlic bulbs used in food processing, is another sclerotial stimulant. In a previous trial, garlic powder effectively reduced sclerotia viability in the upper 5 or 6 inches of the soil profile. However, based on the high incidence of disease in a subsequent garlic crop, garlic powder failed to reduce sclerotia populations below that depth. In this study, garlic powder and DADS were incorporated at a greater depth and applied in a split application to better stimulate germination of sclerotia.

Materials and Methods. This experiment was conducted in a grower field in Kern County. The experimental design was a randomized complete block design with four replications per treatment. Experimental units were 45X100 ft. Data was collected from the center 20X50 ft. Treatments included a nontreated control, garlic powder applied at 200 lbs/acre, diallyl disulfide at 5 mls/m^2 (5.3 gal/acre), and methyl bromide (98% methyl bromide, 2% chloropicrin) at 200 pounds per acre. Half of the garlic powder and diallyl disulfide were applied on Oct. 19, 1999; the other half was applied on Mar. 7, 2000. Stimulant applications were followed by plowing and disking; applications in the spring were followed by mulching. Methyl bromide was applied under tarp by a commercial applicator on Oct. 19, 1999.

Soil populations of sclerotia were determined at the time of soil treatment and at approximately 3-month intervals thereafter. On each sampling date, a composited sample of ten, 1 cm-diameter core subsamples per plot was collected from the soil surface to 6 inches deep. From each sample, 500 cc of soil was directly assayed (or air-dried at room temperature prior to assay) by wet soil sieving. After sieving, soil residue was frozen until observation and viability testing. Sclerotia were identified and plated on water agar amended with 25 ppm streptomycin to observe the proportion from which the fungus grew following disinfestation with mild bleach (0.05% NaOCl) to rid the surface of sclerotia of superficial contaminants. The plots were sampled Oct. 16, 1999, and Feb. 1, April 13, July 7, Oct. 2, and Dec. 1, 2000.

Garlic was planted in the fall, 2001, to evaluate the disease control in the spring of 2002. Treatments (as main plots) included garlic powder at 200 lbs/acre, synthetic garlic oil at 5.3 gal/acre, methyl bromide at 200 lbs/acre, and a nontreated control. Subplots included the following fungicides (applied in the planting furrow at time of planting or as a seed treatment): Folicur (0.4 oz ai/cwt), Folicur (2 l product/ha), Maxim (0.16 oz product/cwt), Moncut (1 lb product/A), Moncut (1 lb product/A at planting and midseason), Serenade (0.5 lb/cwt), and Serenade (10 lb product/A), and a nontreated control. In May, plants were lifted, the tops were cut off, and bulbs were weighed. In addition, general plant health was evaluated on a scale of 0-6, where 0=0%, 1=7%, 2=21%, 3=50%, 4=79%, 5=93%, and 6=100% of the plants affected by white rot.

Results: Populations of sclerotia in soil were high. All treatments significantly reduced sclerotia numbers beginning 2 months after treatment (Fig. 1). Sclerotia viability was most effectively reduced by diallyl disulfide (DADS) and methyl bromide (over 90% mortality). Although garlic powder killed over 80% of the sclerotia, it was less effective than the other treatments. In May, 2002, yields and plant health were estimated (Table 1). Relative to the untreated control, white rot symptoms were reduced and yields were increased by DADS and methyl bromide. Garlic powder did not reduce the incidence of white rot. Among the fungicide treatments, Folicur as a seed dressing and Moncut as a soil drench resulted in the highest yields relative to the control and to the other fungicide treatments. However, the performance of these fungicides may have been due to the control of *Penicillium* and other stand-limiting pathogens.

Discussion: DADS, garlic powder, and methyl bromide reduced populations of sclerotia. DADS approached the effectiveness of methyl bromide. Garlic powder was slightly less effective than the other treatments. The effectiveness of all treatments to reduce the occurrence of white rot was evaluated in May, 2002, in a crop of garlic planted in the fall, 2001. Although garlic powder reduced the viability of sclerotia at about the same magnitude as DADS and methyl bromide, enough sclerotia survived to reduce garlic yields to levels near that in the control plots. Therefore, garlic powder was not as effective as DADS, which resulted in a crop of garlic similar to that which was achieved with methyl bromide. There were also striking visual differences in the plot, i.e., the crops in plots treated with DADS or methyl bromide were clearly healthier than the control or garlic powder-treated plots. The effectiveness of the fungicide treatments was difficult to evaluate. Although there were significant differences among treatments, much of the benefits of the fungicides may have been due to their ability to control seed piece decay and damping-off, since there were no significant differences among the main effects (none, garlic powder, DADS, and methyl bromide). In other words, the relative benefits of the fungicides were similar whether in the control plots, which suffered a relatively high amount of white rot, or in the methyl bromide or DADS-treated plots, which suffered fewer losses from white rot. Whatever their contributions to disease control, Folicur as a seed dressing and Moncut as a soil drench resulted in the highest yields relative to the control and to the other fungicide treatments.

In previous trials, sclerotia below the depth of DADS and garlic powder incorporation survived and caused significant disease and yield loss. For this reason, the current trial was initiated that used better methods to incorporate the materials through the root zone. Results from the earlier trials were encouraging, however, since the degree of sclerotia mortality approached 100% in the treated zone. It is not certain that an economically viable allium crop can follow these sclerotia stimulants. However, it is clear from this research that they have practical use in reducing sclerotia populations in infested fields. This would help prevent movement of sclerotia and contaminated soil to other fields by farm implements, flooding, animals, etc.; thus, DADS and garlic powder are effective substitutes for methyl bromide where growers need to limit spread of white rot. DADS was more effective than garlic powder in these trials.

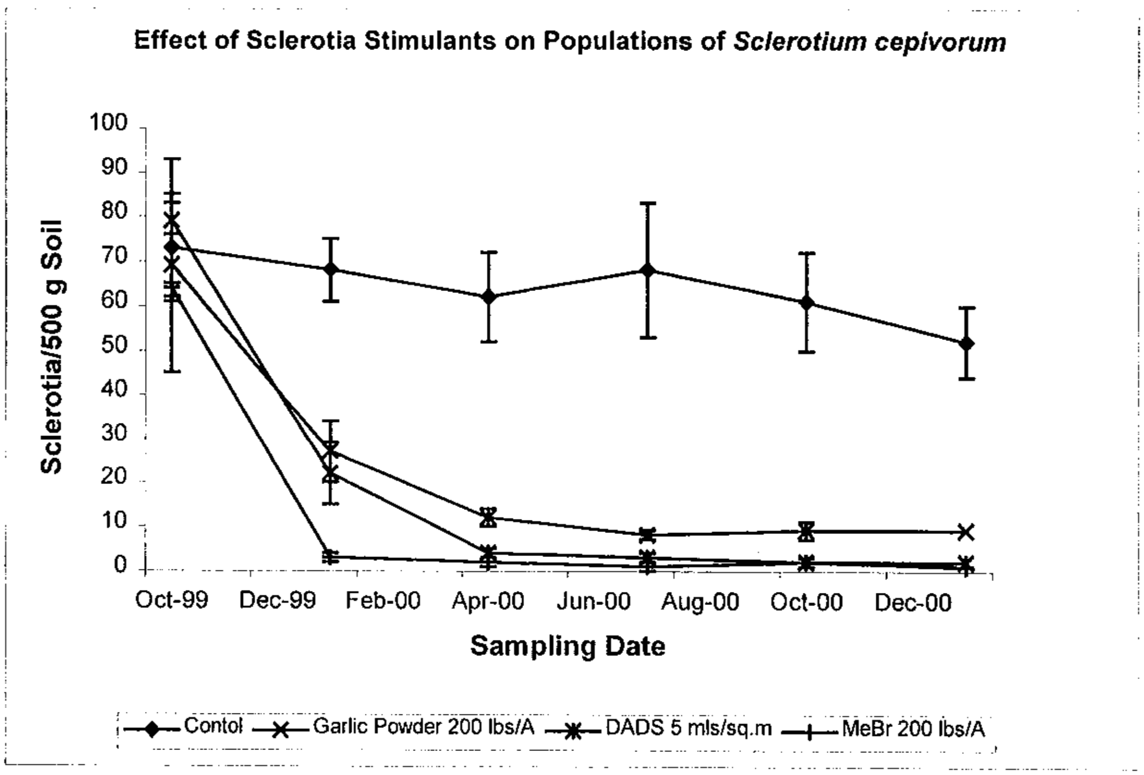
Summary and Conclusions: In this study, germination stimulants of sclerotia of *Sclerotium cepivorum*, including garlic powder and diallyl disulfide, were incorporated into the soil in replicated plots in a commercial field naturally infested with the fungus.

The number of viable sclerotia in the soil treated with DADS and garlic powder was significantly reduced. In soil treated with DADS, the degree of sclerotia mortality achieved approached that achieved with an application of methyl bromide. Garlic powder was only slightly less effective. In a subsequent garlic crop, yields and plant health were greater in the DADS and methyl bromide plots than in the control and garlic powder-treated plots.

In previous trials, sclerotia below the depth of garlic powder incorporation survived and caused significant disease and yield loss as the roots grew below the treated zone. Better incorporation of the sclerotial stimulants down through the soil profile may result in maximum mortality of sclerotia, and therefore disease control.

Appendix

Fig. 1. The effect of germination stimulants and methyl bromide on populations of sclerotia of *Sclerotium cepivorum*



The vertical bars indicate the standard errors of the means.

Table 1. Effect of sclerotia stimulants and fumigation on white rot symptoms and garlic yield.

Treatment	Symptoms index*	Yield (lbs/25 ft)
None	2.0 a	10.32 a
Garlic powder (200 lbs/A)	2.3 a	10.85 a
DADS (5 mls/m^2)	3.5 b	13.40 b
MeBr (200 lbs/A)	4.0 b	14.25 b
LSD, P=0.05	0.77	1.37

*visual rating, 0=all plants dead due to white rot to 5=all plants healthy

Table 2. Effect of fungicide treatments on garlic yield. There was no significant interaction between the main plots (none, garlic powder, DADS, or MeBr) and the subplots (fungicide treatments).

Treatment	Symptom index*	Yield (lbs/10 ft)
None	3.1 a	2.0 a
Folicur soil drench (2 l product/ha)	3.9 ab	2.2 ab
Folicur seed dressing (0.4 oz ai/cwt)	4.7 bc	3.6 d
Maxim seed dressing (0.16 oz product/cwt)	3.8 a	2.8 c
Moncut soil drench (1 lb product/A)	4.7 bc	3.5 d
Moncut soil drench (1 lb product/A at planting and midseason)	5.1 c	3.5 d
Serenade seed dressing (0.5 lb product/cwt)	3.5 a	2.5 abc
Serenade soil drench (10 lb product/A)	3.8 a	2.7 bc
LSD, P=0.05	0.83	0.50

* visual rating where 0=0%, 1=7%, 2=21%, 3=50%, 4=79%, 5=93%, and 6=100% of the plants affected by white rot.